REMARKS

The Pending Claims

Claims 1-5 and 8 are pending and directed to a method for quantitatively detecting an antigen.

Amendments to the Specification and Claims

The specification has been amended to remove obvious typographical, idiomatic or grammatical errors. Claims 6, 7, and 9-21 have been cancelled as drawn to nonelected subject matter. Applicants reserve the right to pursue any canceled subject matter in a continuation, continuation-in-part, divisional application, or other application. Cancellation of any subject matter should not be construed as abandonment of that subject matter. No new matter has been added by way of these amendments.

Summary of the Office Action

The Office has made the restriction requirement final and withdrawn claims 6, 7, and 9-21 from consideration. The Office has objected to the specification for alleged informalities. Claims 1-5 and 8 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Additionally, claims 1-5 and 8 have been rejected under 35 U.S.C. § 103(a) as allegedly obvious over U.S. Patent 5,348,633 (Karger et al.) in view of U.S. Patent 5,630,924 (Fuchs et al.) and Chen et al., *Electrophoresis, 15(1),* 13-21 (1994). The Office has rejected claims 2 and 8 under 35 U.S.C. § 103(a) as allegedly obvious over Karger et al., Fuchs et al., and Chen et al., and further in view of International Published Patent Application WO 89/01974 (Bodmer et al.) and U.S. Patent 4,816,567 (Cabilly et al.). Reconsideration of these rejections is hereby requested.

Discussion of the Objection to the Specification

The Office has objected to the specification for alleged informalities. Applicants have amended the specification to remove obvious typographical, idiomatic, or grammatical errors. Accordingly, the objection to the specification should be withdrawn.

Discussion of the Indefiniteness Rejection

The Office has rejected claims 1-5 and 8 as allegedly indefinite for the stated reason that the claims do not recite a step relating detecting fluorescence to quantitatively detecting an antigen. Applicants respectfully traverse this rejection for the following reasons.

The claims are clear to those skilled in the art. Those skilled in the art would readily understand that after carrying out the step of detecting the fluorescence of the immune complex, quantitative detection can be performed, for example, in view of the teachings in the specification. Accordingly, the rejection is erroneous and should be withdrawn.

Discussion of the Obviousness Rejections

The Office has rejected claims 1-5 and 8 as allegedly obvious over the combination of several references. These rejections are traversed for the following reasons.

The present invention represented by the pending claims is predicated on the need for a method of quantitatively detecting an antigen, which method enables the analysis of the antigen with high accuracy, even when the isoelectric point of the antigen as the analyte is close to that of a fluorescently labeled antibody used in the method (see, e.g., page 7, line 23, through page 8, line 3, of the specification). Applicants discovered that it is possible to analyze an antigen with high accuracy by using an Fab' antibody having a uniform isoelectric point, which is modified by adding an amino acid sequence comprising a charged amino acid residue and labeling the Fab' antibody with a fluorescent dye, wherein the Fab' antibody forms an immune complex with an antigen in an analytical sample (see, page 8, lines 4-10, of the specification). The addition of the amino acid sequence comprising a charged amino acid residue to the Fab' antibody alters the isoelectric point of the fluorescent labeled Fab' antibody. This alteration of the isoelectric point of the Fab' antibody makes it possible to separate and detect complexes formed by an antigen and antibody when the isoelectric points of the Fab' antibody and antigen are the same or close to the same.

The method of producing the Fab' antibody having a uniform isoelectric point comprises (a) providing an Fd chain gene encoding a VH region and CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab' antibody, and an L chain gene encoding the L chain of the Fab' antibody; (b) linking the Fd chain gene and the L chain gene in the expressible state to obtain a gene expressing an Fab' antibody; (c) modifying the gene expressing an Fab' antibody to express and amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain, and site-specifically mutating in the gene expressing an Fab' antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a gene expressing a charge modified Fab' antibody; (d) transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain

an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal CH1 region, and (e) binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric point obtained in the fourth step.

In contrast to the present invention, Karger et al. teaches a complicated method of forming fluorescent-labeled Fab' fragments having uniform isoelectric points, which isoelectric points are dictated by the IgG produced by the hybridoma (see, e.g., Figure 2 of Karger et al.). Karger et al. does not teach or suggest modifying the isoelectric point of the Fab' antibody, e.g., by the methods disclosed in the present invention. Unlike the method of the present invention represented by the pending claims, using the method disclosed in Karger et al. does not allow the separation and detection of antigen/antibody complexes when the isoelectric points of the antigen and Fab' fragment are the same or close to the same.

The Office contends that Fuchs et al. discloses methods of labeling and charge modification of monoclonal antibody fragments. Fuchs et al. describes the alteration of the charge of the antibody by bonding Fab' fragments and charged amino acid polymers (e.g., polyglutamic acid (poly-Glu)) or oligonucleotides (e.g., polyT20) (see, e.g., column 11, of Fuchs et al.). However, as described in Fuchs et al., the number of amino acids in the charged amino acid sequence polymer is not fixed, and only the rough molecular weight is mentioned (see, e.g., columns 11-12, of Fuchs et al.). For example, Fuchs et al. discloses that the poly-Glu to be attached to an Fab' antibody fragment is available from Sigma (St. Louis, Mo.) at various degrees of polymerization and with a relatively narrow molecular weight distribution (see, e.g., column 12, lines 48-54, of Fuchs et al.). As set forth in Exhibit 1, a search of Sigma-Aldrich website for "polyglutamic acid" yielded 11 results, all of which have molecular weights of spanning large ranges (e.g., poly-L-glutamic acid has a molecular weight range from 2,000-15,000). For this reason, the antibody with the addition of the charged amino acid polymer of Fuchs et al. cannot be considered to have a uniform charge or uniform isoelectric point, as required by the pending claims. Furthermore, the Fab' fragment of Fuchs et al. does not have a fluorescent label and charged molecule bound to the Fab' fragment at the same time, also as recited in the pending claims.

To use the Fab' fragment having a uniform isoelectric point while maintaining the uniformity of isoelectric points and fluorescent labeling of the Fab' fragment, the number and the position of the fluorescent dye binding has to be controlled. The bonding of the Fd chain and L chain of the Fab' fragment depends on disulfide bonding. The Karger and Fuchs

patents describe a method of using sulfide groups for fluorescent labeling, but use a complicated process including oxygen treatment, reduction reaction, and oxidizing treatment using IgG as a starting material, wherein the number of sulfide groups of cysteine has to be fixed (see, e.g., Figure 2, of Karger et al.).

The pending application solves the above-described problem by using a genetic engineering method in which an Fab' fragment containing one cysteine, which cysteine is not involved with the binding of the Fd chain and L chain, is formed. By forming complexes of antigens having nonuniform isoelectric points with fluorescently labeled Fab' fragments having uniform isoelectric points, and separating the two by isoelectric-focusing, the isoelectric points of individual complexes reflect the isoelectric points of individual antigens. It is important in this scenario, to use an Fab' fragment which makes the complex monovalent with the binding antigen. Namely, when the binding is with an antigen such a IgG or $F(ab')_2$, which makes the complex divalent, then the complex formed becomes different from a monovalent one (formed by an Fab' fragment), and changes the isoelectric point. This makes the separation and detection of the complex very difficult because different types of binding result in different isoelectric points and result in different effects on the complexes.

Chen et al. discloses the effective separation of antigens, antibodies, and complexes of an antigen and antibody by electrophoresis, especially by capillary electrophoresis. Additionally, Chen et al. discloses that the separation can be improved by the addition of certain charge-bearing organic molecules, such as synthetic oligonucleotides (see, page 14, paragraph bridging columns 1 and 2, of Chen et al.). However, the addition of charge-bearing organic molecules in Chen et al. is to an antigen, and not to an Fab' antibody as recited in the pending claims (see, e.g., page 19, paragraph bridging columns 1 and 2, of Chen et al.). This differs from the method of the present invention, in which Fab' fragments are fluorescently labeled and have uniform isoelectric points. In the present invention, uniformity of isoelectric point is maintained when the isoelectric point is altered, and antigen, antibody, and the complex of both are effectively separated by electrophoresis (e.g., capillary electrophoresis). Chen et al. hypothesizes about fluorescently labeling an antibody, but does not specify that the antibody is an Fab' fragment or teach or suggest that the antibody has a uniform isoelectric point, which are required by the pending claims (see, e.g., page 21, column 2, last paragraph, of Chen et al.). Thus, one of ordinary skill in the art would not be motivated to combine the disclosure of Chen et al. with that of Karger et al. and/or Fuchs et al., and even if one of ordinary skill in the art were to combine the disclosures, he would not arrive at the present invention represented by the pending claims.

Therefore, the pending claims cannot be considered to be obvious in view of the combination of the Karger, Fuchs, and Chen references. Furthermore, Bodmer et al. and Cabilly et al. do not provide the additional motivation or missing aspects of the pending claims, such that one of ordinary skill in the art would arrive at the present invention from the combination of references.

Bodmer et al. refers to a genetic engineering method of forming an antibody wherein the number of sulfide groups of cysteine are controlled. However, this is not relevant to the Fab' fragment disclosed in present application. Even if the IgG and $F(ab')_2$ of Bodmer et al. contain one sulfide group of cysteine at the hinging part, the method still requires complicated fragmentation processing which involves oxygen processing, reduction processing, and the like, similar to the processes disclosed in the Karger and Fuchs patents discussed above. Thus, given the disclosure of Bodmer et al., the formation of fluorescence-labeled Fab' fragments having uniform isoelectric points as recited in the pending claims could not easily done by one of ordinary skill in the art.

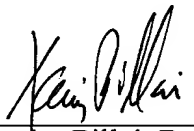
Cabilly et al. is related to the improvement of antibody molecules (e.g., human-type antibody molecules) using a genetic engineering method. In the method disclosed in Cabilly et al., amino acids in the antibody are replaced, especially the amino acids in the CH1 region, so that an amino acid sequence becomes the same as that of a human antibody molecule (see, e.g., column 5, lines 22-35, of Cabilly et al.). While the present invention involves the conversion of amino acids at the CH1 area (i.e., to form Fab' fragments having uniform isoelectric points) by a genetic engineering method, the actual method and the reason for the conversion of amino acids is completely different than that of Cabilly et al. Specifically, the method of the present invention involves causing a specific mutation on at least one codon encoding an amide group-containing amino acid residue in the CH1 region to result in a codon encoding an amide group-non-containing amino acid residue except for cysteine to correct a nonuniform isoelectric point. The method of the present invention leads to the production of an Fab' fragment without amino acids that cause the nonuniformity of isoelectric points (i.e., an Fab' fragment with a uniform isoelectric point). Accordingly, given the different reasons for mutating amino acids in the CH1 region disclosed in Cabilly et al. and the present invention, one of ordinary skill in the art would not have been motivated to modify the disclosures of Karger et al., Fuchs et al., Chen et al., and/or Bodmer et al., based on the teachings of Cabilly et al. to arrive at the present invention.

For the above reasons, the method of quantitatively detecting an antigen recited in the pending claims would not be obvious in view of the combination of cited references, and the rejections should be withdrawn.

Conclusion

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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Product Number	Description
81326	**Poly-L-glutamic acid**, BioChemika 2,000-15,000 25513-46-6, MFCD00148021
P1370	**Poly-D-glutamic acid sodium salt**, mol wt 15,000-25,000 30811-79-1, MFCD00166407
P4033	**Poly-D-glutamic acid sodium salt**, mol wt 15,000-50,000 30811-79-1, MFCD00166407
P9917	**Poly-D-glutamic acid sodium salt**, mol wt 2,000-15,000 30811-79-1, MFCD00166407
81327	**Poly-L-glutamic acid sodium salt**, BioChemika 15,000-50,000 26247-79-0, MFCD00148021
81328	**Poly-L-glutamic acid sodium salt**, BioChemika 50,000-100,000 26247-79-0, MFCD00148021
P1818	**Poly-L-glutamic acid sodium salt**, mol wt 1,500-5,000 26247-79-0, MFCD00081868
P4761	**Poly-L-glutamic acid sodium salt**, mol wt 15,000-50,000 26247-79-0, MFCD00081868
P4636	**Poly-L-glutamic acid sodium salt**, mol wt 3,000-15,000 26247-79-0, MFCD00081868
P4886	**Poly-L-glutamic acid sodium salt**, mol wt 50,000-100,000 26247-79-0, MFCD00081868
P1943	**Poly-L-glutamic acid sodium salt**, mol wt 750-4,000 26247-79-0, MFCD00081868

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